Get a jump ahead on your Western blots and gels

80-6448-41/Rev. B1/09-99

amersham pharmacia biotech

You said, "Versatility!" We say "Processor Plus!"

The new Hoefer™ Processor Plus from Amersham Pharmacia Biotech will help you get the jump on both Western blot processing and acrylamide gel staining. One intelligent fluid delivery system handles both applications. Changing from one task to the other is as simple as switching trays. Gel staining or Western blot processing—it's your choice. Load the reagents, select a programmed protocol, and push "Start"—the Processor Plus will fill and empty the tray with the right solution at the right time. Now you're free to jump on more challenging and productive tasks.

Change processing mode with a fast and simple switch of a tray

Westerns: high sensitivity chemiluminescent ECL,™ ECL Plus, or chromogenic protocols

Staining: Coomassie™ blue or high-sensitivity silver staining for both protein and nucleic acid

Programmable control of solution, volume, processing time, and waste container

Proven design for consistent, high-quality performance, every time

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Blot Processor:

- Trays for four mini- or two standard-sized blots at one time
- Five preprogrammed and five user-defined blot protocols for flexibility
- Tray and lid design separate fluid delivery and removal to avoid cross-contamination
- Automatic delivery of antibodies down to 10 ml; pause for manual addition and recovery of expensive antibodies

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The Blot Processor and Gel Stainer Share:

- Rapid delivery and removal of reagents via high-speed peristaltic pump
- Smooth and even rocking of the tray for thorough fluid mixing and coverage
- Straightforward protocol entry and editing, up to 30 steps
- Removable Protocol Key to store and protect critical protocols
- 10-port valve for multiple reagent protocols and hazardous waste segregation

Gel Stainer:

- Mini and standard/large-format tray sizes
- Nine preprogrammed and four user-defined protocols for proteins and nucleic acids
- Rhomboidal-shaped trays for efficient mixing with minimal volumes
- Reagent volumes variable from 125 to 400 ml
- High-strength coated magnets hold gels in place

Step by Step:

Blot Processor

1

Place blots into tray chambers and cover. Set up antibody solutions in conical tubes; wash reagents in bottles.

2

Turn valves on to select required chambers.

3

Select your protocol, enter number of blots and tray size and push "Start".

Preprogrammed Protocols

Blot processing

ECL Plus
ECL
Standard Chromogenic
Enhanced Chromogenic
Clean

Blot processing tray capacity

mini-gels (up to 9 × 9.5 cm)	4
standard (up to 16 × 16 cm)	2

4

Leave the Processor Plus to add, mix and remove reagents according to the selected protocol.

5

Apply ECL detection reagent to membrane manually, or add chromogenic reagents automatically.

Step by Step:

Gel Stainer

1

Prepare staining reagents; insert numbered tubes into appropriate reagent and waste bottles.

2

Place the gel into the tray and cover with the glass lid.

3

Select your protocol. Enter reagent volume and tray size and push “Start”.

Preprogrammed Protocols

DNA silver stain (1)
Protein silver stain (3)
Protein Coomassie stain (4)

Staining tray capacity

Gel size (cm)	Tray size 19 × 29 cm	29 × 35 cm
mini (8 × 7)	4	6
standard (14 × 16, SE 600)	1	2
large (12.5 × 26, ExcelGel™, CleanGel™)	1	2

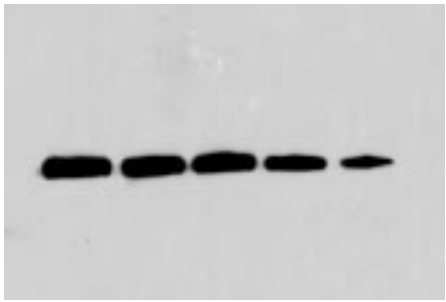
4

Take stained gel out of the tray.

5

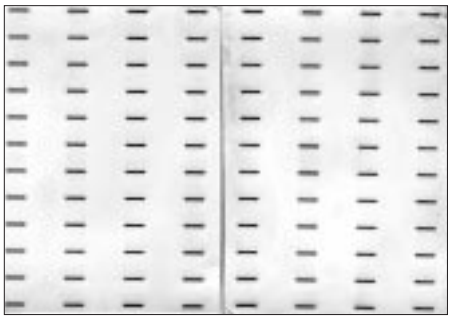
Dispose of waste materials.

Blot processing results



ECL Plus Detection

Dilutions of an *E. coli* extract were analysed for GroEL with ECL Plus detection. After separation on 12% SDS acrylamide gel, proteins were transferred onto Hybond™ P membrane in a Hoefer™ TE 22 Tank Transfer Unit. Amounts of extract loaded range from 5.8 to 1.15 µg of total protein. Processor Plus blot protocol #1 was used with rabbit anti-GroEL antibody, Biotinylated Goat Anti-Rabbit IgG (H+L) (RPN480), Streptavidin-Alkaline Phosphatase Conjugate (RPN1234), and detection reagents from the ECL Plus kit.

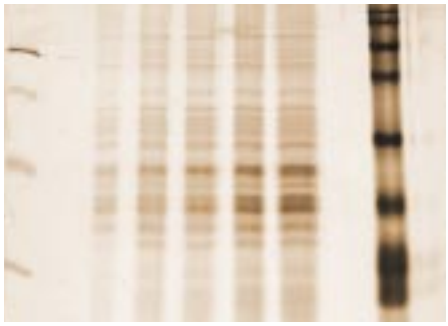


A B

Manual or Automated?

Bovine serum albumin (25 ng per well) was slot blotted onto Hybond C pure nitrocellulose. The BSA was detected with rabbit anti-BSA, Biotinylated Goat Anti-Rabbit IgG (H+L) Antibody (RPN480), and Streptavidin-Alkaline Phosphatase Conjugate (RPN1234). The chromogenic substrate was BCIP/NBT. Membrane A was processed in the Processor Plus, and membrane B was processed manually.

A Immunodetection: Detection of gliadin proteins in wheat seed extracts



B Silver staining: Detection of total protein in wheat seed extracts

Blot processing *and* staining

Duplicate SDS acrylamide gel separations of Chinese Spring wheat seed gliadin proteins were prepared. (A) Immunodetection: One gel was blotted onto Hybond C pure nitrocellulose membrane using the Hoefer miniVE Electrophoresis Unit, then developed with Processor Plus blot processing protocol #4 (primary antibody: rabbit anti-gliadin; detection with biotinylated goat anti-rabbit IgG (H+L) antibody, streptavidin-alkaline phosphatase conjugate, BCIP/NBT substrate). (B) Gel staining: Gel was stained to detect total protein on Processor Plus using staining protocol #2 with the PlusOne™ Silver Staining Kit, Protein

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Tips for 2D Gels

Blotting

- Use Rainbow markers as MW markers for confirmation of transfer on the blot.
- After visualization of the target protein, all proteins on the blot can be stained using an AuroDye Forte Kit for colloidal gold staining.
- When using ECL Plus Detection, use ECL Western Blotting chemluminescent MW markers to provide both MW calibration and gel orientation.

Staining

- Clean gloves should be used for handling gels and equipment. Wash out silver staining tray with soapy water after each use.
- Cut the corners of the 2-D gels or place labels within the gel for orientation and identification.
- An extra 30 minute fix step helps reduce background due to presence of carrier ampholytes in 2-D gels.
- De-staining protein samples prior to MALDI/TOF mass spec analysis may enhance sensitivity. Gharahdaghi, *et al Electrophoresis* **20**, 601-605 (1999)

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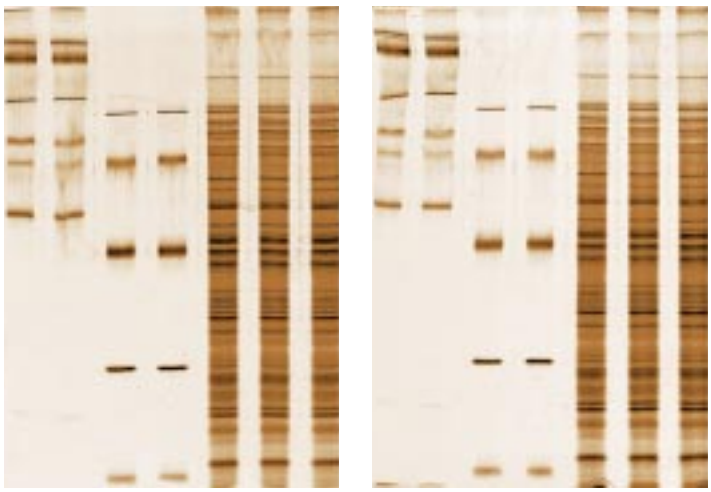
2D Results

Immunoblot of cell culture extract of glioblastoma multiform brain tumor cells. The blot was incubated with mouse anti-p53 primary antibody (1:30,000) and then goat anti-mouse horseradish peroxidase conjugate (1:5,000). Immunodetection was performed with the ECL detection kit. The human cell culture extract and monoclonal antibody were generously provided by Dr. Mike Harrington, Huntington Medical Research Institute, Pasadena, CA.

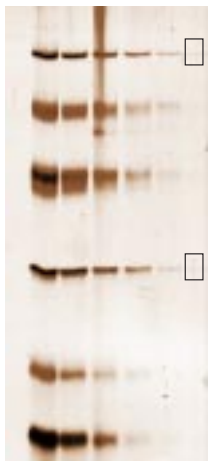
2-D gel of a cell culture extract of glioblastoma multiform brain tumor cells stained with PusOne Silver Kit, Protein, using silver staining protocol 2 of the Processor Plus. Protein detected on the 2-D blot are boxed in yellow.

Gel Staining results



Reproducibility of staining between gels

Two 1-mm-thick gels were loaded and run identically with *E. coli* extract. The gels were then stained in two different units, using solutions from the same PlusOne Silver Staining Kit, Protein (left).



Lane: 6

Sensitivity of protein staining

A 12.5% ExcelGel™ was loaded with serial dilutions of Amersham Pharmacia Biotech High Molecular Weight SDS standards and stained using the PlusOne Silver Staining Kit, Protein, at 24 °C. The faintest bands that can be seen are phosphorylase b (~0.22 ng) and carbonic anhydrase (~0.29 ng) in lane 6. As is true with most gel staining techniques, limits of sensitivity are somewhat protein specific.

Lane: 6

Sensitivity of DNA staining

A GeneGel Clean (15%) was loaded with serial dilutions of ΦX 174 DNA *Hae* III digest. The gel was stained using the PlusOne DNA Silver Staining Kit. The faintest DNA band that can be seen is at 118 bp in lane 6 (15 pg).

Ordering Information

Hoefer Processor Plus 80-6444-04

Base unit, reagent tubing, protocol key (order tray pack separately)

Blot processing tray options

Blot Processing Tray Pack 80-6444-23
Complete with tray base, disposable mini and standard trays, lid, reagent bottles and rack, and waste bottle

Gel staining tray options

Staining Tray Pack 19 × 29 cm 80-6444-80
Complete with gel staining tray base, PTFE-coated tray, lid

Staining Tray Pack 29 × 35 cm 80-6445-18
Complete with gel staining tray base, PTFE-coated tray, lid

Accessories for blot processing

Blot Processing Mini Tray 80-6444-42
Disposable mini trays (3/pk)

Blot Processing Standard Tray 80-6444-61
Disposable standard trays (3/pk)

ECL Western blotting reagents

For 1,000 cm^2 membrane	RPN 2109
For 2,000 cm^2 membrane	RPN 2209
For 4,000 cm^2 membrane	RPN 2106
For 6,000 cm^2 membrane	RPN 2134
ECL Western Blotting Analysis System	RPN 2108

ECL Plus Western blotting reagents

For 1,000 cm^2 membrane	RPN 2132
For 3,000 cm^2 membrane	RPN 2133

Accessories for gel staining

Staining tray 19 × 29 cm	80-6444-99
Staining tray 29 × 35 cm	80-6445-37

Staining Kits

PlusOne DNA Silver Staining Kit	17-6000-30
PlusOne Silver Staining Kit, Protein	17-1150-01
Coomassie tablets (40), PhastGel Blue	17-0518-01

For more information, visit the Amersham Pharmacia Biotech Web site:

www.apbiotech.com

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